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L1: Entry 1 of 1

File: USPT

May 11, 2004

US-PAT-NO: 6734004

DOCUMENT-IDENTIFIER: US 6734004 B2

TITLE: Modified phytases

DATE-ISSUED: May 11, 2004

## INVENTOR-INFORMATION:

| NAME               | CITY            | STATE | ZIP CODE | COUNTRY |
|--------------------|-----------------|-------|----------|---------|
| Kostrewa; Dirk     | Freiburg        |       |          | DE      |
| Pasamontes; Luis   | Trimbach        |       |          | CH      |
| Tomschy; Andrea    | Grenzach-Wyhlen |       |          | DE      |
| van Loon; Adolphus | Rheinfelden     |       |          | CH      |
| Vogel; Kurt        | Basel           |       |          | CH      |
| Wyss; Markus       | Liestal         |       |          | CH      |

US-CL-CURRENT: [435/196](#); [435/252.3](#), [435/320.1](#), [435/913](#), [435/916](#), [435/917](#), [536/23.2](#)

## CLAIMS:

What is claimed is:

1. A polynucleotide comprising a DNA sequence coding for a modified *Aspergillus fumigatus* phytase with a specific activity improved over the specific activity of the corresponding unmodified *Aspergillus fumigatus* phytase wherein the amino acid sequence of the unmodified phytase has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* (SEQ ID NO:1), as identified by PILEUP version 8 amino acid sequence alignment program, to an amino acid selected from the group consisting of Ala, Val, Leu, Ile, Thr, Gly, and Asn.
2. A polynucleotide according to claim 1 wherein the modified *Aspergillus fumigatus* phytase further comprises an additional mutation selected from the group consisting of S66D, S140Y, D141G, A205E, Q274L, G277D, G277K, Y282H, and N340S.
3. A polynucleotide comprising a DNA sequence coding for a modified *Aspergillus fumigatus* phytase with a specific activity improved over the specific activity of the corresponding unmodified *Aspergillus fumigatus* phytase wherein the amino acid sequence of the modified *Aspergillus fumigatus* phytase has a mutation selected from the group consisting of S66D, S140Y, D141G, A205E, Q274L, G277D, G277K, Y282H, N340S, and combinations thereof, wherein the respective amino acid position of each mutation corresponds to the amino acid position of an *Aspergillus niger* phytase (SEQ ID NO:1) as identified by PILEUP version 8 amino acid alignment program.

4. A vector comprising the polynucleotide of claim 1.
5. The vector of claim 4 which is an expression vector.
6. A host cell which has been transformed by a polynucleotide of claim 1.
7. A host cell which has been transformed by a vector of claim 4.
8. A polynucleotide according to claim 1 wherein the unmodified phytase has the sequence of SEQ ID NO:3.
9. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Ala.
10. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Val.
11. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Leu.
12. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Ile.
13. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Thr.
14. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Asn.
15. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Gly.
16. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q23L and S62D.
17. A polynucleotide according to claim 8 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q23L, S136Y, and D137G.
18. A polynucleotide according to claim 3 wherein the unmodified phytase has the sequence of SEQ ID NO:3.
19. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: S62D.
20. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: S136Y.

21. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: D137G.
22. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: A200E.
23. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q269L.
24. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: G272D.
25. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: G272K.
26. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Y277H.
27. A polynucleotide according to claim 18 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: N335S.
28. A vector comprising the polynucleotide of claim 3.
29. The vector of claim 28 which is an expression vector.
30. A host cell which has been transformed by a polynucleotide of claim 3.
31. A host cell which has been transformed by a vector of claim 28.

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L2: Entry 4 of 4

File: USPT

May 21, 2002

US-PAT-NO: 6391605DOCUMENT-IDENTIFIER: US 6391605 B1**\*\* See image for Certificate of Correction \*\***

TITLE: Modified phytases

DATE-ISSUED: May 21, 2002

## INVENTOR-INFORMATION:

| NAME               | CITY            | STATE | ZIP CODE | COUNTRY |
|--------------------|-----------------|-------|----------|---------|
| Kostrewa; Dirk     | Freiburg        |       |          | DE      |
| Pasamontes; Luis   | Trimbach        |       |          | CH      |
| Tomschy; Andrea    | Grenzach-Wyhlen |       |          | DE      |
| van Loon; Adolphus | Rheinfelden     |       |          | CH      |
| Vogel; Kurt        | Basel           |       |          | CH      |
| Wyss; Markus       | Liestal         |       |          | CH      |

US-CL-CURRENT: 435/196; 424/94.6

## CLAIMS:

What is claimed is:

1. A modified *Aspergillus fumigatus* phytase with a specific activity improved over the specific activity of the corresponding unmodified *Aspergillus fumigatus* phytase wherein the amino acid sequence of the unmodified phytase has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* (SEQ ID NO:1), as identified by PILEUP version 8 amino acid sequence alignment program, to an amino acid selected from the group consisting of Ala, Val, Leu, Ile, Thr, Gly, and Asn.

2. A modified *Aspergillus fumigatus* phytase according to claim 1, further comprising an additional mutation selected from the group consisting of S66D, S140Y, D141G, A205E, Q274L, G277D, G277K, Y282H, and N340S.

3. A modified *Aspergillus fumigatis* phytase with a specific activity improved over the specific activity of the corresponding unmodified *Aspergillus fumigatus* phytase wherein the amino acid sequence of the modified *Aspergillus fumigatus* phytase has a mutation selected from the group consisting of S66D, S140Y, D141G, A205E, Q274L, G277D, G277K, Y282H, N340S, and combinations thereof, wherein the respective amino acid position of each mutation corresponds to the amino acid position of an *Aspergillus niger* phytase (SEQ ID NO:1) as identified by PILEUP version 8 amino acid alignment program.

4. A modified phytase according to claim 1 wherein the unmodified phytase has the sequence of SEQ ID NO:3.

5. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Ala.

6. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Val.

7. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Leu.

8. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Ile.

9. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Thr.

10. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Asn.

11. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been changed at a position corresponding to position 27 of the phytase of *Aspergillus niger* to the amino acid Gly.

12. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q23L and S62D.

13. A modified phytase according to claim 4 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q23L, S136Y, and D137G.

14. A modified phytase according to claim 3 wherein the unmodified phytase has the sequence of SEQ ID NO:3.

15. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: S62D.

16. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: S136Y.

17. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: D137G.

18. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: A200E.

19. A modified phytase according to claim 3 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Q269L.

20. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: G272D.

- 21. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: G272K.
- 22. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: Y277H.
- 23. A modified phytase according to claim 14 wherein the amino acid sequence of SEQ ID NO:3 has been modified as follows: N335S.
- 24. A food or feed composition comprising a modified phytase of claim 1.

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L2: Entry 3 of 4

File: USPT

Jun 17, 2003

US-PAT-NO: 6579975

DOCUMENT-IDENTIFIER: US 6579975 B1

TITLE: Consensus phytases

DATE-ISSUED: June 17, 2003

INVENTOR-INFORMATION:

| NAME            | CITY      | STATE | ZIP CODE | COUNTRY |
|-----------------|-----------|-------|----------|---------|
| Lehmann; Martin | Inzlingen |       |          | DE      |

US-CL-CURRENT: [536/23.2](#); [435/195](#), [435/196](#), [435/252.3](#), [435/320.1](#), [435/325](#), [435/69.1](#)

CLAIMS:

What is claimed is:

1. A polynucleotide encoding a consensus protein of SEQ ID NO:2.
2. A polynucleotide encoding a consensus protein of SEQ ID NO:1.
3. A polynucleotide which encodes a consensus protein having the amino acid sequence of SEQ ID NO:2 except that Q at position 50 has been replaced by L, T, or G.
4. A polynucleotide which encodes a consensus protein having the amino acid sequence of SEQ ID NO:2 except that Q at position 50 has been replaced by T and Y at position 51 has been replaced by N.
5. A polynucleotide which encodes a consensus protein having the amino acid sequence of SEQ ID NO:2 except that Q at position 50 has been replaced by L and Y at position 51 has been replaced by N.

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☐ 1. Document ID: US 20040175757 A1

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L2: Entry 1 of 11

File: PGPB

Sep 9, 2004

PGPUB-DOCUMENT-NUMBER: 20040175757

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040175757 A1

TITLE: Low allergenic protein variants

PUBLICATION-DATE: September 9, 2004

INVENTOR-INFORMATION:

| NAME                | CITY        | STATE | COUNTRY | RULE-47 |
|---------------------|-------------|-------|---------|---------|
| Olsen, Arne Agerlin | Virum       |       | DK      |         |
| Roggen, Erwin Lugo  | Lyngby      |       | DK      |         |
| Ernst, Steffen      | Kobenhavn N |       | DK      |         |

US-CL-CURRENT: [435/7.1](#); [436/518](#)

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KIMC | Draw D |
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☐ 2. Document ID: US 20040142424 A1

L2: Entry 2 of 11

File: PGPB

Jul 22, 2004

PGPUB-DOCUMENT-NUMBER: 20040142424

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040142424 A1

TITLE: Modified phytases

PUBLICATION-DATE: July 22, 2004

INVENTOR-INFORMATION:

| NAME               | CITY            | STATE | COUNTRY | RULE-47 |
|--------------------|-----------------|-------|---------|---------|
| Kostrewa, Dirk     | Freiburg        |       | DE      |         |
| Pasamontes, Luis   | Trimbach        |       | CH      |         |
| Tomschy, Andrea    | Grenzach-Wyhlen |       | DE      |         |
| Loon, Adolphus van | Rheinfelden     |       | CH      |         |
| Vogel, Kurt        | Basle           |       | CH      |         |



Wyss, Markus

Liestal

CH

US-CL-CURRENT: 435/69.1; 435/196, 435/320.1, 435/325, 435/455, 536/23.2

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|----------|
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☐ 3. Document ID: US 20040126844 A1

L2: Entry 3 of 11

File: PGPB

Jul 1, 2004

PGPUB-DOCUMENT-NUMBER: 20040126844

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040126844 A1

TITLE: Using mutations to improve aspergillus phytases

PUBLICATION-DATE: July 1, 2004

## INVENTOR-INFORMATION:

| NAME                | CITY        | STATE | COUNTRY | RULE-47 |
|---------------------|-------------|-------|---------|---------|
| Lei, Xingen         | Ithaca      | NY    | US      |         |
| Mullaney, Edward J. | New Orleans | LA    | US      |         |
| Ullah, Abul H.J.    | Slidell     | LA    | US      |         |

US-CL-CURRENT: 435/69.1; 435/196, 435/320.1, 435/419, 536/23.2

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|----------|
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☐ 4. Document ID: US 20030208788 A1

L2: Entry 4 of 11

File: PGPB

Nov 6, 2003

PGPUB-DOCUMENT-NUMBER: 20030208788

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030208788 A1

TITLE: Phytase variants

PUBLICATION-DATE: November 6, 2003

## INVENTOR-INFORMATION:

| NAME                  | CITY     | STATE | COUNTRY | RULE-47 |
|-----------------------|----------|-------|---------|---------|
| Matsui, Tomoko        | Chiba    |       | JP      |         |
| Fuglsang, Claus Crone | Vekso    |       | DK      |         |
| Svendsen, Allan       | Horsholm |       | DK      |         |
| Fukuyama, Shiro       | Chiba    |       | JP      |         |

US-CL-CURRENT: 800/278; 435/196, 800/8

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|----------|
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☐ 5. Document ID: US 20030190677 A1

L2: Entry 5 of 11

File: PGPB

Oct 9, 2003

PGPUB-DOCUMENT-NUMBER: 20030190677

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030190677 A1

TITLE: Consensus phytases

PUBLICATION-DATE: October 9, 2003

## INVENTOR-INFORMATION:

| NAME            | CITY      | STATE | COUNTRY | RULE-47 |
|-----------------|-----------|-------|---------|---------|
| Lehmann, Martin | Inzlingen |       | DE      |         |

US-CL-CURRENT: 435/7.1; 702/19

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. De |
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☐ 6. Document ID: US 20030119066 A1

L2: Entry 6 of 11

File: PGPB

Jun 26, 2003

PGPUB-DOCUMENT-NUMBER: 20030119066

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030119066 A1

TITLE: Diagnostic kit for detecting immunogenic response and method of screening

PUBLICATION-DATE: June 26, 2003

## INVENTOR-INFORMATION:

| NAME                   | CITY     | STATE | COUNTRY | RULE-47 |
|------------------------|----------|-------|---------|---------|
| Roggen, Erwin Ludo     | Lyngby   |       | DK      |         |
| Nilsson, Nina Teeres   | Kavlinge |       | SE      |         |
| Ernst, Steffen         | Bronshoj |       | DK      |         |
| Patkar, Shamkant Anant | Lyngby   |       | DK      |         |
| Friis, Esben Peter     |          |       | US      |         |

US-CL-CURRENT: 435/7.1; 424/185.1, 435/7.93

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. De |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|----------|
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☐ 7. Document ID: US 20030092155 A1

L2: Entry 7 of 11

File: PGPB

May 15, 2003

PGPUB-DOCUMENT-NUMBER: 20030092155  
PGPUB-FILING-TYPE: new  
DOCUMENT-IDENTIFIER: US 20030092155 A1

TITLE: Modified phytases

PUBLICATION-DATE: May 15, 2003

INVENTOR-INFORMATION:

| NAME               | CITY            | STATE | COUNTRY | RULE-47 |
|--------------------|-----------------|-------|---------|---------|
| Kostrewa, Dirk     | Freiburg        |       | DE      |         |
| Pasamontes, Luis   | Trimbach        |       | CH      |         |
| Tomschy, Andrea    | Grenzach-Wyhlen |       | DE      |         |
| Loon, Adolphus van | Rheinfelden     |       | CH      |         |
| Vogel, Kurt        | Basle           |       | CH      |         |
| Wyss, Markus       | Liestal         |       | CH      |         |

US-CL-CURRENT: 435/195; 424/94.6, 435/188, 435/196

|      |       |          |       |        |                |      |           |           |             |        |      |        |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|--------|
| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|--------|

☐ 8. Document ID: US 6734004 B2

L2: Entry 8 of 11

File: USPT

May 11, 2004

US-PAT-NO: 6734004  
DOCUMENT-IDENTIFIER: US 6734004 B2

TITLE: Modified phytases

DATE-ISSUED: May 11, 2004

INVENTOR-INFORMATION:

| NAME               | CITY            | STATE | ZIP CODE | COUNTRY |
|--------------------|-----------------|-------|----------|---------|
| Kostrewa; Dirk     | Freiburg        |       |          | DE      |
| Pasamontes; Luis   | Trimbach        |       |          | CH      |
| Tomschy; Andrea    | Grenzach-Wyhlen |       |          | DE      |
| van Loon; Adolphus | Rheinfelden     |       |          | CH      |
| Vogel; Kurt        | Basel           |       |          | CH      |
| Wyss; Markus       | Liestal         |       |          | CH      |

US-CL-CURRENT: 435/196; 435/252.3, 435/320.1, 435/913, 435/916, 435/917, 536/23.2

|      |       |          |       |        |                |      |           |           |             |        |      |        |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|--------|
| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|--------|

☐ 9. Document ID: US 6720174 B1

L2: Entry 9 of 11

File: USPT

Apr 13, 2004

US-PAT-NO: 6720174  
DOCUMENT-IDENTIFIER: US 6720174 B1

TITLE: Phytases

DATE-ISSUED: April 13, 2004

INVENTOR-INFORMATION:

| NAME            | CITY      | STATE | ZIP CODE | COUNTRY |
|-----------------|-----------|-------|----------|---------|
| Lehmann; Martin | Princeton | NJ    |          |         |

US-CL-CURRENT: 435/196; 435/18, 435/195, 530/350, 536/23.2

|      |       |          |       |        |                |      |           |           |             |        |      |        |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|--------|
| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|--------|

☐ 10. Document ID: US 6686164 B1

L2: Entry 10 of 11

File: USPT

Feb 3, 2004

US-PAT-NO: 6686164  
DOCUMENT-IDENTIFIER: US 6686164 B1

TITLE: Low allergenic protein variants

DATE-ISSUED: February 3, 2004

INVENTOR-INFORMATION:

| NAME                | CITY        | STATE | ZIP CODE | COUNTRY |
|---------------------|-------------|-------|----------|---------|
| Olsen; Arne Agerlin | Kaplevej    |       |          | DK      |
| Roggen; Erwin Lugo  | Lyngby      |       |          | DK      |
| Ernst; Steffen      | Kobenhavn N |       |          | DK      |

US-CL-CURRENT: 435/7.1; 435/DIG.15, 435/DIG.4, 436/501, 436/513

|      |       |          |       |        |                |      |           |           |             |        |      |        |
|------|-------|----------|-------|--------|----------------|------|-----------|-----------|-------------|--------|------|--------|
| Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D |
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| <input type="checkbox"/> | L9  | phytase? and 3-D structure                     | 3         |
| <input type="checkbox"/> | L8  | phytase? with 3-D structure                    | 0         |
| <input type="checkbox"/> | L7  | modified phytase? with 3-D structure           | 0         |
| <input type="checkbox"/> | L6  | modified phytase? with 3-dimensional structure | 0         |
| <input type="checkbox"/> | L5  | modified phytase? and 3-dimensional structure  | 0         |
| <input type="checkbox"/> | L4  | 3-Dimensional structure and phytase            | 9         |
| <input type="checkbox"/> | L3  | 3-Dimensional structure and phytase.clm.       | 4         |
| <input type="checkbox"/> | L2  | 3D structure and phytase.clm.                  | 11        |
| <input type="checkbox"/> | L1  | 3D structure with phytase.clm.                 | 0         |

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=> s three-dimensional structure and enzyme?  
 L1 10237 THREE-DIMENSIONAL STRUCTURE AND ENZYME?

=> dup rem l1  
 PROCESSING IS APPROXIMATELY 12% COMPLETE FOR L1  
 PROCESSING IS APPROXIMATELY 30% COMPLETE FOR L1  
 PROCESSING IS APPROXIMATELY 51% COMPLETE FOR L1  
 PROCESSING IS APPROXIMATELY 72% COMPLETE FOR L1  
 PROCESSING IS APPROXIMATELY 89% COMPLETE FOR L1  
 PROCESSING COMPLETED FOR L1  
 L2 4139 DUP REM L1 (6098 DUPLICATES REMOVED)

=> s l2 and phytase?  
 L3 9 L2 AND PHYTASE?

=> d l3 1-9 ibib ab

L3 ANSWER 1 OF 9 MEDLINE on STN  
 ACCESSION NUMBER: 2004165683 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 15060628  
 TITLE: The role of disulfide bonds in the conformational stability  
 and catalytic activity of **phytase**.  
 AUTHOR: Wang Xiao-Yun; Meng Fan-Guo; Zhou Hai-Meng  
 CORPORATE SOURCE: College of Life Science, Shandong Agricultural University,  
 Shandong Tai'an, People's Republic of China.  
 SOURCE: Biochemistry and cell biology = Biochimie et biologie  
 cellulaire, (2004 Apr) 82 (2) 329-34.  
 Journal code: 8606068. ISSN: 0829-8211.  
 PUB. COUNTRY: Canada  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200411  
 ENTRY DATE: Entered STN: 20040403  
 Last Updated on STN: 20041219  
 Entered Medline: 20041124

AB Previous studies have predicted five disulfide bonds in *Aspergillus niger*  
**phytase** (phy A). To investigate the role of disulfide bonds,  
 intrinsic fluorescence spectra, far-ultraviolet circular dichroism (CD)  
 spectra, and an **enzyme** activity assay were used to compare the  
 differences of catalytic activity and conformational stability of

**phytase** during denaturation in urea in the presence and absence of dithiothreitol (DTT). In the presence of 2 mM DTT, the inactivation and unfolding were greatly enhanced at the same concentration of denaturant. The fluorescence emission maximum red shift and decreases of ellipticity at 222 nm were in accord with the changes of catalytic activity. The kinetics of the unfolding courses were a biphasic process consisting of two first-order reactions in the absence of DTT and a monophasic process of a first-order reaction in the presence of DTT. The results suggested that the loss of enzymatic activity was most likely because of a conformational change, and that disulfide bonds played an important role in **three-dimensional structure** and catalytic activity.

L3 ANSWER 2 OF 9 MEDLINE on STN  
 ACCESSION NUMBER: 2001081457 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 10933495  
 TITLE: Optimization of the catalytic properties of *Aspergillus fumigatus* **phytase** based on the **three-dimensional structure**.  
 AUTHOR: Tomschy A; Tessier M; Wyss M; Brugger R; Broger C; Schnoebelen L; van Loon A P; Pasamontes L  
 CORPORATE SOURCE: F. Hoffmann-La Roche Ltd, Basel, Switzerland.  
 SOURCE: Protein science : a publication of the Protein Society, (2000 Jul) 9 (7) 1304-11.  
 Journal code: 9211750. ISSN: 0961-8368.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200101  
 ENTRY DATE: Entered STN: 20010322  
 Last Updated on STN: 20010322  
 Entered Medline: 20010109

AB Previously, we determined the DNA and amino acid sequences as well as biochemical and biophysical properties of a series of fungal **phytases**. The amino acid sequences displayed 49-68% identity between species, and the catalytic properties differed widely in terms of specific activity, substrate specificity, and pH optima. With the ultimate goal to combine the most favorable properties of all **phytases** in a single protein, we attempted, in the present investigation, to increase the specific activity of *Aspergillus fumigatus* **phytase**. The crystal structure of *Aspergillus niger* NRRL 3135 **phytase** known at 2.5 Å resolution served to specify all active site residues. A multiple amino acid sequence alignment was then used to identify nonconserved active site residues that might correlate with a given favorable property of interest. Using this approach, Gln27 of *A. fumigatus* **phytase** (amino acid numbering according to *A. niger* **phytase**) was identified as likely to be involved in substrate binding and/or release and, possibly, to be responsible for the considerably lower specific activity (26.5 vs. 196 U x [mg protein]<sup>-1</sup>) at pH 5.0) of *A. fumigatus* **phytase** when compared to *Aspergillus terreus* **phytase**, which has a Leu at the equivalent position. Site-directed mutagenesis of Gln27 of *A. fumigatus* **phytase** to Leu in fact increased the specific activity to 92.1 U x (mg protein)<sup>-1</sup>, and this and other mutations at position 27 yielded an interesting array of pH activity profiles and substrate specificities. Analysis of computer models of **enzyme**-substrate complexes suggested that Gln27 of wild-type *A. fumigatus* **phytase** forms a hydrogen bond with the 6-phosphate group of myo-inositol hexakisphosphate, which is weakened or lost with the amino acid substitutions tested. If this hydrogen bond were indeed responsible for the differences in specific activity, this would suggest product release as the rate-limiting step of the *A. fumigatus* wild-type **phytase** reaction.

L3 ANSWER 3 OF 9 MEDLINE on STN

ACCESSION NUMBER: 1998007872 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 9349716  
 TITLE: Cloning of the **phytases** from *Emericella nidulans* and the thermophilic fungus *Talaromyces thermophilus*.  
 AUTHOR: Pasamontes L; Haiker M; Henriquez-Huecas M; Mitchell D B; van Loon A P  
 CORPORATE SOURCE: F. Hoffmann-La Roche Ltd., Vitamins and Fine Chemicals Division, Basel, Switzerland.. luis.pasamontes@roche.com  
 SOURCE: Biochimica et biophysica acta, (1997 Sep 12) 1353 (3) 217-23.  
 Journal code: 0217513. ISSN: 0006-3002.  
 PUB. COUNTRY: Netherlands  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 OTHER SOURCE: GENBANK-U59802; GENBANK-U59803  
 ENTRY MONTH: 199711  
 ENTRY DATE: Entered STN: 19971224  
 Last Updated on STN: 19990129  
 Entered Medline: 19971119

AB **Phytases** (EC 3.1.3.8) belong to the family of histidine acid phosphatases. We have cloned the **phytases** of the fungi *Emericella nidulans* and *Talaromyces thermophilus*. The putative **enzyme** encoded by the *E. nidulans* sequence consists of 463 amino acids and has a Mr of 51785. The protein deduced from the *T. thermophilus* sequence consists of 466 amino acids corresponding to a Mr of 51450. Both predicted amino acid sequences exhibited high identity (48% to 67%) to known **phytases**. This high level of identity allowed the modelling of all available fungal **phytases** based on the **three-dimensional structure** coordinates of the *Aspergillus niger* **phytase**. By this approach we identified 21 amino acids which are conserved in fungal phyA **phytases** and are part of the residues forming the substrate pocket. Furthermore, potential glycosylation sites were identified and compared between the aforementioned **phytases** and the *A. niger* **phytase**.

L3 ANSWER 4 OF 9 MEDLINE on STN  
 ACCESSION NUMBER: 97032764 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 8878514  
 TITLE: Disulfide bonds are necessary for structure and activity in *Aspergillus ficuum* **phytase**.  
 AUTHOR: Ullah A H; Mullaney E J  
 CORPORATE SOURCE: Southern Regional Research Center, ARS, USDA, New Orleans, Louisiana 70124, USA.  
 SOURCE: Biochemical and biophysical research communications, (1996 Oct 14) 227 (2) 311-7.  
 Journal code: 0372516. ISSN: 0006-291X.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199612  
 ENTRY DATE: Entered STN: 19970128  
 Last Updated on STN: 19980206  
 Entered Medline: 19961203

AB The function of disulfide bonds in *Aspergillus ficuum* **phytase** was elucidated by unfolding studies, using guanidinium hydrochloride (Gu.HCl) as denaturant. Although the **enzyme** is totally inactivated by 0.8 M Gu.HCl, at pH 5.0, the active conformation is instantaneously restored by 0.6 M Gu.HCl, at pH 5.0. Conditions which would permit refolding of **phytase** are completely negated by 10 mM beta-mercaptoethanol and causes its catalytic demise at pH 7.5. Assay of free thiols using Ellman's reagent indicates that none of the thiols in the ten cysteines in **phytase** are free; five disulfide bonds were predicted for the **enzyme**. Sequence comparison of mold



**phytases** and yeast acid phosphatases indicates four conserved cysteines. Thus, disulfide bonds play an important role in the folding of fungal **phytase**; any perturbation of the process of its formation causes an altered **three-dimensional structure** that is inconsistent with catalytic activity.

L3 ANSWER 5 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2005:37339 HCAPLUS  
TITLE: Structure and function of Escherichia coli  
glucose-1-phosphatase  
AUTHOR(S): Lee, Daniel C.; Jia, Zongchao  
CORPORATE SOURCE: Department of Biochemistry, Queen's University,  
Kingston, ON, K7L 3N6, Can.  
SOURCE: Recent Research Developments in Molecular Biology  
(2003), 1, 251-261  
CODEN: RRDMKT  
PUBLISHER: Research Signpost  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The E. coli glucose-1-phosphatase, a member of the histidine acid phosphatase family, serves primarily as a glucose scavenger in the bacterial periplasm with high activity for glucose-1-phosphate. The **enzyme** also possesses unique substrate specificities for various inositol phosphates, such as being a 3 and only-3 **phytase**. Detn. of the **three-dimensional structure** of E. coli glucose-1-phosphatase would facilitate the understanding of such unique phytate and glucose-1-phosphate specificities of the **enzyme**. The crystal structure of E. coli glucose-1-phosphatase has been detd. at 2.6-.ANG. resoln. by the method of multi-wavelength anomalous dispersion using a tungstate deriv., together with the complex structure with glucose-1-phosphate and a mutant structure both at 2.4-.ANG. resoln. Lacking substrate-induced conformational change, the active-site pocket of glucose-1-phosphatase is both rigid and small. The presence of two unique gating residues, Glu196 and Leu24, together with the conserved features of histidine acid phosphatase active site, is responsible for the unique substrate selectivity of phytate and glucose-1-phosphate, as well as the unusually high pH range of glucose-1-phosphate activity. Based on the structural characterization, comparison and modeling, simple structural principles were developed that not only accurately predict hydrolysis products of all inositol phosphates and explain substrate specificity of glucose-1-phosphatase, but also rationalize similar general catalytic characteristics across the histidine acid phosphatase family.

REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 6 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2001:50782 HCAPLUS  
DOCUMENT NUMBER: 134:82721  
TITLE: A 2.1 .ANG. crystal structure of a novel thermostable **phytase** from Bacillus amyloliquefaciens  
INVENTOR(S): Oh, Tae Kwang; Oh, Byung Chul; Ha, Nam Chul; Choi, Yang Woong; Lee, Dong Kyu; Oh, Byung Ha  
PATENT ASSIGNEE(S): Korea Institute of Bioscience and Biotechnology, S. Korea; Daesung Microbiological Labs. Co., Ltd.  
SOURCE: PCT Int. Appl., 17 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

| PATENT NO.    | KIND | DATE     | APPLICATION NO. | DATE     |
|---------------|------|----------|-----------------|----------|
| WO 2001004275 | A1   | 20010118 | WO 2000-KR2     | 20000104 |
| W: JP, US     |      |          |                 |          |

RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,  
PT, SE

KR 2001009461 A 20010205 KR 1999-27826 19990709  
EP 1194530 A1 20020410 EP 2000-900932 20000104

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,  
IE, FI

JP 2003504047 T2 20030204 JP 2001-509479 20000104  
US 2002142433 A1 20021003 US 2002-43623 20020109  
US 6808909 B2 20041026

PRIORITY APPLN. INFO.: KR 1999-27826 A 19990709  
WO 2000-KR2 W 20000104

AB The present invention relates to a thermostable **phytase** from *Bacillus amyloliquefaciens* DS-11 with a 2.1 .ANG. crystal structure of a propeller type comprising six external blades that encompass the outer boundary of the crystal and six internal calcium-binding sites that are embedded inside the above crystal. Each of the six blades again consists of 4 or 5 anti-parallel .beta.-strands, and the six calcium binding sites consist of 3 high-affinity calcium binding sites and 3 low-affinity binding sites, which are involved in the **enzyme's** thermostability and catalytic activity, resp. The above-mentioned Ca<sup>2+</sup> binding motifs are expected to be utilized in synthesizing highly thermostable proteins and the elucidation of active sites of an **enzyme** from a **three-dimensional structure** can help to design new **enzymes** having those sites with the aid of recent advanced technol. of protein engineering.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 7 OF 9 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

ACCESSION NUMBER: 2000:187018 SCISEARCH

THE GENUINE ARTICLE: 289EQ

TITLE: From DNA sequence to improved functionality: using protein sequence comparisons to rapidly design a thermostable consensus **phytase**

AUTHOR: Lehmann M (Reprint); Kostrewa D; Wyss M; Brugger R; Darcy A; Pasamontes L; vanLoon A P G M

CORPORATE SOURCE: F HOFFMANN LA ROCHE & CO LTD, GRENZACHERSTR 124, CH-4070 BASEL, SWITZERLAND (Reprint)

COUNTRY OF AUTHOR: SWITZERLAND

SOURCE: PROTEIN ENGINEERING, (JAN 2000) Vol. 13, No. 1, pp. 49-57. Publisher: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.  
ISSN: 0269-2139.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 43

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Naturally-occurring **phytases** having the required level of thermostability for application in animal feeding have not been found in nature thus far. We decided to de novo construct consensus **phytases** using primary protein sequence comparisons. A consensus **enzyme** based on 13 fungal **phytase** sequences had normal catalytic properties, but showed an unexpected 15-22 degrees C increase in unfolding temperature compared with each of its parents. As a first step towards understanding the molecular basis of increased heat resistance, the crystal structure of consensus **phytase** was determined and compared with that of *Aspergillus niger* **phytase**. *Aspergillus niger* **phytase** unfolds at much lower temperatures. In most cases, consensus residues were indeed expected, based on comparisons of both three-dimensional structures, to contribute more to **phytase** stabilization than non-consensus amino acids. For some consensus amino acids, predicted by structural comparisons to destabilize the protein, mutational analysis was performed. Interestingly, these consensus residues

in fact increased the unfolding temperature of the consensus **phytase**. In summary, for fungal **phytases** apparently an unexpected direct link between protein sequence conservation and protein stability exists.

L3 ANSWER 8 OF 9 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN  
ACCESSION NUMBER: 2005:12185 BIOSIS  
DOCUMENT NUMBER: PREV200500019770  
TITLE: Thermostable **phytase** with 2.1 ANG . . . crystal structure.  
AUTHOR(S): Oh, Tae Kwang [Inventor, Reprint Author]; Ha, Nam Chul [Inventor]; Oh, Byung Ha [Inventor]  
CORPORATE SOURCE: Daejeon, South Korea  
ASSIGNEE: Korea Research Institute of Bioscience and Biotechnology, Daejeon, South Korea; Daesung Microbiological Labs. Co., Kyungki-do, South Korea  
PATENT INFORMATION: US 6808909 October 26, 2004  
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Oct 26 2004) Vol. 1287, No. 4.  
<http://www.uspto.gov/web/menu/patdata.html>. e-file.  
ISSN: 0098-1133 (ISSN print).  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
ENTRY DATE: Entered STN: 22 Dec 2004  
Last Updated on STN: 22 Dec 2004

AB The present invention relates to a thermostable **enzyme** with a 2.1 ANG crystal structure of a propeller type comprising six external blades that encompass the outer boundary of the crystal and six internal calcium-binding sites that are embedded inside the above crystal. Each of the six blades comprises 4 or 5 anti-parallel beta-strands, and the six calcium binding sites consist of 3 high-affinity calcium binding sites and 3 low-affinity binding sites, which are involved in the **enzyme's** thermostability and catalytic activity, respectively. The above-mentioned Ca<sup>2+</sup> binding motifs are expected to be utilized in synthesizing highly thermostable proteins and the elucidation of active sites of an **enzyme** from a three-dimensional structure can help to design new **enzymes** having those sites with the aid of recent advanced technology of protein engineering.

L3 ANSWER 9 OF 9 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation. on STN  
ACCESSION NUMBER: 2003:438331 BIOSIS  
DOCUMENT NUMBER: PREV200300438331  
TITLE: Fungal phyA gene expressed in potato leaves produces active and stable **phytase**.  
AUTHOR(S): Ullah, Abul H. J. [Reprint Author]; Sethumadhavan, Kandan; Mullaney, Edward J.; Ziegelhoffer, Thomas; Austin-Phillips, Sandra  
CORPORATE SOURCE: Southern Regional Research Center, ARS, USDA, 1100 Robert E. Lee Boulevard, New Orleans, LA, 70124, USA  
[aullah@srrc.ars.usda.gov](mailto:aullah@srrc.ars.usda.gov)  
SOURCE: Biochemical and Biophysical Research Communications, (June 27 2003) Vol. 306, No. 2, pp. 603-609. print.  
CODEN: BBRCA9. ISSN: 0006-291X.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
ENTRY DATE: Entered STN: 24 Sep 2003  
Last Updated on STN: 24 Sep 2003

AB Fungal phyA gene from *Aspergillus ficum* (niger) was cloned and expressed in potato leaves. The recombinant **enzyme** was stable and catalytically active. The expressed protein in the leaves of the dicotyledonous plant retained most physical and catalytic properties of the benchmark *A. ficum* **phytase**. The expressed **enzyme** was, however, 15% less glycosylated than the native **phytase**. The usual bi-hump pH optima profile, which is characteristic of the fungal **phytase**, was altered; however, the pH optimum at 5.0 was unchanged

for phytate and at 4.0 for synthetic substrate p-nitrophenyl phosphate. The temperature was, however, unchanged. The expressed **phytase** was found to be as sensitive as the native **enzyme** to the inhibitory action of pseudo substrate, myo-inositol hexasulfate, while losing about 90% of the activity at 20  $\mu$ M inhibitor concentration. Similar to the benchmark **phytase**, the expressed **phytase** in leaves was completely inactivated by Arg modifier phenylglyoxal at 60 nM. In addition, the expressed **phytase** in the leaves was inhibited by antibody raised against a 20-mer internal peptide, which is present on the surface of the molecule as shown by the X-ray deduced 3D structure of fungal **phytase**. Taken together, the biochemical evidences indicate that fungal **phytase** when cloned and expressed in potato leaves produces a stable and active biocatalyst. 'Biofarming,' therefore, is an alternative way to produce functional hydrolytic **enzymes** as exemplified by the expression of A. ficuum (niger) phyA gene in potato leaf.

=> log y

COST IN U.S. DOLLARS

SINCE FILE

TOTAL

ENTRY

SESSION

FULL ESTIMATED COST

37.04

37.25

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)

SINCE FILE

TOTAL

ENTRY

SESSION

CA SUBSCRIBER PRICE

-1.46

-1.46

STN INTERNATIONAL LOGOFF AT 16:37:20 ON 02 FEB 2005

=> d l1 1-10

L1 ANSWER 1 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN  
RN 793185-78-1 REGISTRY  
CN 3-Phytase (*Idiomarina loihiensis* strain L2TR) (9CI) (CA INDEX NAME)

OTHER NAMES:

CN GenBank AAV80945  
CN GenBank AAV80945 (Translated from: GenBank AE017340)  
FS PROTEIN SEQUENCE  
MF Unspecified  
CI MAN  
SR GenBank  
LC STN Files: CA, CAPLUS  
DT.CA Caplus document type: Journal  
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*

\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\*  
1 REFERENCES IN FILE CA (1907 TO DATE)  
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 2 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN  
RN 746497-03-0 REGISTRY  
CN Phytase (*Bacillus licheniformis* strain ATCC 14580 gene phy) (9CI) (CA INDEX NAME)

OTHER NAMES:

CN GenBank AAU22048  
CN GenBank AAU22048 (Translated from: GenBank CP000002)  
FS PROTEIN SEQUENCE  
MF Unspecified  
CI MAN  
SR GenBank  
LC STN Files: CA, CAPLUS  
DT.CA Caplus document type: Journal  
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*

\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\*  
1 REFERENCES IN FILE CA (1907 TO DATE)  
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 3 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN  
RN 736053-29-5 REGISTRY  
CN Histidine acid phosphatase / 6-phytase (*Yersinia pseudotuberculosis* strain IP32953) (9CI) (CA INDEX NAME)

OTHER NAMES:

CN GenBank CAH21659  
CN GenBank CAH21659 (Translated from: GenBank BX936398)  
FS PROTEIN SEQUENCE  
MF Unspecified  
CI MAN  
SR GenBank  
LC STN Files: CA, CAPLUS  
DT.CA Caplus document type: Journal  
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*

\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\*  
1 REFERENCES IN FILE CA (1907 TO DATE)  
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 4 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN  
RN 703422-65-5 REGISTRY  
CN Phosphatase, phytate (*Sartorya fumigata* strain ATCC-32239 precursor) (9CI)

(CA INDEX NAME)

OTHER NAMES:

CN **Phytase (Sartorya fumigata strain ATCC-32239 precursor)**  
FS PROTEIN SEQUENCE  
MF Unspecified  
CI MAN  
SR CA  
LC STN Files: CA, CAPLUS  
DT.CA Caplus document type: Journal  
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*

\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\*

1 REFERENCES IN FILE CA (1907 TO DATE)

1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 5 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN  
RN 703422-64-4 REGISTRY  
CN **Phosphatase, phytate (Aspergillus fumigatus strain ATCC-58128 precursor)**  
(9CI) (CA INDEX NAME)

OTHER NAMES:

CN **Phytase (Aspergillus fumigatus strain ATCC-58128 precursor)**  
FS PROTEIN SEQUENCE  
MF Unspecified  
CI MAN  
SR CA  
LC STN Files: CA, CAPLUS  
DT.CA Caplus document type: Journal  
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*

\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\*

1 REFERENCES IN FILE CA (1907 TO DATE)

1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 6 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN  
RN 703422-63-3 REGISTRY  
CN **Phosphatase, phytate (Aspergillus fumigatus strain ATCC-32722 precursor)**  
(9CI) (CA INDEX NAME)

OTHER NAMES:

CN **Phytase (Aspergillus fumigatus strain ATCC-32722 precursor)**  
FS PROTEIN SEQUENCE  
MF Unspecified  
CI MAN  
SR CA  
LC STN Files: CA, CAPLUS  
DT.CA Caplus document type: Journal  
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*

\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\*

1 REFERENCES IN FILE CA (1907 TO DATE)

1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 7 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN  
RN 703422-62-2 REGISTRY  
CN **Phosphatase, phytate (Aspergillus fumigatus strain ATCC-26906 precursor)**  
(9CI) (CA INDEX NAME)

OTHER NAMES:

CN **Phytase (Aspergillus fumigatus strain ATCC-26906 precursor)**  
FS PROTEIN SEQUENCE  
MF Unspecified  
CI MAN  
SR CA  
LC STN Files: CA, CAPLUS

DT.CA Caplus document type: Journal  
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*  
\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\*  
1 REFERENCES IN FILE CA (1907 TO DATE)  
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 8 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN  
RN 703422-61-1 REGISTRY  
CN Phosphatase, phytate (Aspergillus fumigatus strain ATCC-36934 precursor)  
(9CI) (CA INDEX NAME)

OTHER NAMES:

CN Phytase (Aspergillus fumigatus strain ATCC-34625 precursor)  
FS PROTEIN SEQUENCE  
MF Unspecified  
CI MAN  
SR CA  
LC STN Files: CA, CAPLUS

DT.CA Caplus document type: Journal  
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

\*\*RELATED SEQUENCES AVAILABLE WITH SEQLINK\*\*

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*  
\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\*  
1 REFERENCES IN FILE CA (1907 TO DATE)  
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 9 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN  
RN 685165-68-8 REGISTRY  
CN Phosphatase, phytate (synthetic fungi isoenzyme consensus phytase-12  
gene fcp12 precursor) (9CI) (CA INDEX NAME)

OTHER NAMES:

CN 169: PN: US6599735 SEQID: 169 claimed protein  
FS PROTEIN SEQUENCE  
MF Unspecified  
CI MAN  
SR CA  
LC STN Files: CA, CAPLUS, USPATFULL

DT.CA Caplus document type: Patent  
RL.P Roles from patents: BIOL (Biological study); PREP (Preparation); PRP  
(Properties)

\*\*RELATED SEQUENCES AVAILABLE WITH SEQLINK\*\*

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*  
\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\*  
1 REFERENCES IN FILE CA (1907 TO DATE)  
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 10 OF 99 REGISTRY COPYRIGHT 2005 ACS on STN  
RN 685165-67-7 REGISTRY  
CN DNA (synthetic fungi gene fcp7 phytate phosphatase isoenzyme  
consensus phytase-7-specifying) (9CI) (CA INDEX NAME)

OTHER NAMES:

CN 168: PN: US6599735 SEQID: 168 claimed DNA  
FS NUCLEIC ACID SEQUENCE  
MF Unspecified  
CI MAN  
SR CA  
LC STN Files: CA, CAPLUS, USPATFULL

DT.CA Caplus document type: Patent  
RL.P Roles from patents: BIOL (Biological study); PREP (Preparation); PRP  
(Properties)

**\*\*RELATED SEQUENCES AVAILABLE WITH SEQLINK\*\***

**\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\***

**\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\***

1 REFERENCES IN FILE CA (1907 TO DATE)

1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

=> s (phytase or acid phosphatase)

99 PHYTASE

6903830 ACID

19756 PHOSPHATASE

559 ACID PHOSPHATASE

(ACID(W) PHOSPHATASE)

L2 657 (PHYTASE OR ACID PHOSPHATASE)

=> s phytase and acid phosphatase

99 PHYTASE

6903830 ACID

19756 PHOSPHATASE

559 ACID PHOSPHATASE

(ACID(W) PHOSPHATASE)

L3 1 PHYTASE AND ACID PHOSPHATASE

=> d l3

L3 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2005 ACS on STN

RN 736053-29-5 REGISTRY

CN **Histidine acid phosphatase / 6-phytase (Yersinia pseudotuberculosis strain IP32953) (9CI) (CA INDEX NAME)**

OTHER NAMES:

CN GenBank CAH21659

CN GenBank CAH21659 (Translated from: GenBank BX936398)

FS PROTEIN SEQUENCE

MF Unspecified

CI MAN

SR GenBank

LC STN Files: CA, CAPLUS

DT.CA Caplus document type: Journal

RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

**\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\***

**\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\***

1 REFERENCES IN FILE CA (1907 TO DATE)

1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

=> s acid phosphatase

6903830 ACID

19756 PHOSPHATASE

L4 559 ACID PHOSPHATASE

(ACID(W) PHOSPHATASE)

=> d l4 1-5

L4 ANSWER 1 OF 559 REGISTRY COPYRIGHT 2005 ACS on STN

RN 800285-54-5 REGISTRY

CN **Acid phosphatase SurE (Silicibacter pomeroyi strain DSS-3 gene surE) (9CI) (CA INDEX NAME)**

OTHER NAMES:

CN GenBank AAV95933

CN GenBank AAV95933 (Translated from: GenBank CP000031)

FS PROTEIN SEQUENCE

MF Unspecified



CI MAN  
SR GenBank  
LC STN Files: CA, CAPLUS  
DT.CA Caplus document type: Journal  
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*  
\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\*  
1 REFERENCES IN FILE CA (1907 TO DATE)  
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L4 ANSWER 2 OF 559 REGISTRY COPYRIGHT 2005 ACS on STN  
RN 793202-58-1 REGISTRY  
CN **Type II phosphatidic acid phosphatase (Idiomarina loihiensis strain L2TR) (9CI) (CA INDEX NAME)**

OTHER NAMES:

CN GenBank AAV82625  
CN GenBank AAV82625 (Translated from: GenBank AE017340)  
FS PROTEIN SEQUENCE  
MF Unspecified  
CI MAN  
SR GenBank  
LC STN Files: CA, CAPLUS  
DT.CA Caplus document type: Journal  
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*  
\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\*  
1 REFERENCES IN FILE CA (1907 TO DATE)  
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L4 ANSWER 3 OF 559 REGISTRY COPYRIGHT 2005 ACS on STN  
RN 793201-16-8 REGISTRY  
CN **PAP2 (acid phosphatase) superfamily membrane protein (Idiomarina loihiensis strain L2TR) (9CI) (CA INDEX NAME)**

OTHER NAMES:

CN GenBank AAV82483  
CN GenBank AAV82483 (Translated from: GenBank AE017340)  
FS PROTEIN SEQUENCE  
MF Unspecified  
CI MAN  
SR GenBank  
LC STN Files: CA, CAPLUS  
DT.CA Caplus document type: Journal  
RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*  
\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\*  
1 REFERENCES IN FILE CA (1907 TO DATE)  
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L4 ANSWER 4 OF 559 REGISTRY COPYRIGHT 2005 ACS on STN  
RN 786740-43-0 REGISTRY  
CN **DNA (human clone DE10316701-SEQID-259 gene ACP1 soluble acid phosphatase 1 cDNA plus flanks) (9CI) (CA INDEX NAME)**

OTHER NAMES:

CN 47: PN: DE10316701 PAGE: 1026 claimed DNA  
FS NUCLEIC ACID SEQUENCE  
MF Unspecified  
CI MAN  
SR CA  
LC STN Files: CA, CAPLUS, TOXCENTER  
DT.CA Caplus document type: Patent  
RL.P Roles from patents: BIOL (Biological study); PRP (Properties); USES (Uses)

\*\*RELATED SEQUENCES AVAILABLE WITH SEQLINK\*\*

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*

\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\*

1 REFERENCES IN FILE CA (1907 TO DATE)

1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L4 ANSWER 5 OF 559 REGISTRY COPYRIGHT 2005 ACS on STN

RN 775172-47-9 REGISTRY

CN Acid phosphatase SurE (Haloarcula marismortui strain ATCC 43049 gene  
surE) (9CI) (CA INDEX NAME)

OTHER NAMES:

CN GenBank AAV45535

CN GenBank AAV45535 (Translated from: GenBank AY596297)

FS PROTEIN SEQUENCE

MF Unspecified

CI MAN

SR GenBank

LC STN Files: CA, CAPLUS

DT.CA CPlus document type: Journal

RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties)

\*\*\* STRUCTURE DIAGRAM IS NOT AVAILABLE \*\*\*

\*\*\* USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE \*\*\*

1 REFERENCES IN FILE CA (1907 TO DATE)

1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

=> log y

COST IN U.S. DOLLARS

SINCE FILE

TOTAL

ENTRY

SESSION

FULL ESTIMATED COST

73.85

74.06

STN INTERNATIONAL LOGOFF AT 11:50:41 ON 03 FEB 2005

=> s (3-dimentional structure? or 3D structure?) phytase and aspergillus) and (mutant? or m  
MISSING OPERATOR RUCTURE?) PHYTASE  
The search profile that was entered contains terms or  
nested terms that are not separated by a logical operator.

=> s (3-dimentional structure? or 3D structure?) and phytase and aspergillus) and (mutant?  
UNMATCHED RIGHT PARENTHESIS 'SPERGILLUS) AND'  
The number of right parentheses in a query must be equal to the  
number of left parentheses.

=> s (3-dimentional structure? or 3D structure?) and phytase and aspergillus) and (mutant?  
UNMATCHED RIGHT PARENTHESIS 'SPERGILLUS) AND'  
The number of right parentheses in a query must be equal to the  
number of left parentheses.

=> s (3-dimentional structure? or 3D structure?) and (phytase and aspergillus) and (mutant?  
L1 1 (3-DIMENTIONAL STRUCTURE? OR 3D STRUCTURE?) AND (PHYTASE AND  
ASPERGILLUS) AND (MUTANT? OR MODIFIED OR VARIANT? OR MUTAION?)

=> d l1 ibib ab

L1 ANSWER 1 OF 1 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 1999-01149 BIOTECHDS  
TITLE: New **modified** protease with increased activity;  
recombinant enzyme production by vector expression in host  
cell and application in food industry  
AUTHOR: Kostrewa D; Pasamontes L; Tomschy A; van Loon A; Vogel K;  
Wyss M  
PATENT ASSIGNEE: Roche  
LOCATION: Basle, Switzerland.  
PATENT INFO: AU 9859543 1 Oct 1998  
APPLICATION INFO: AU 1998-59543 25 Mar 1998  
PRIORITY INFO: EP 1997-810175 25 Mar 1997  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 1998-595453 [51]

AB A process for the production of a **modified phytase**  
with improved activity properties is new and involves: modelling the  
three-dimensional (3D) **structure** of the  
**phytase** (preferably from *Aspergillus fumigatus*) being  
**modified**, and optionally that of another **phytase**  
(preferably from *Aspergillus terreus* or *Aspergillus*  
*niger*) with better activity, on the basis of the 3D-  
**structure** of *Aspergillus niger* **phytase**;  
identifying the amino acids in the active site that differ between the  
two structures; changing at least one of the differing amino acids in the  
active site in the **phytase** and incorporating the DNA molecule  
into an expression vector; and using the DNA or the vector to transform a  
host cell which is then cultured and isolated. Also claimed are: the  
**phytase** produced; vectors containing the **modified**  
**phytase** DNA; host cells containing the DNA or vector; and feed or  
foods containing the **modified phytase**. Phytases are  
useful as feed additives for converting **phytase** to myoinositol  
and inorganic phosphate. The addition of a **modified**  
**phytase** to feed reduces the amount of phytate in animal manure.  
(118pp)

=> s (3-dimentional structure? or 3D structure?) and phytase and (mutant? or modified or v  
L2 1 (3-DIMENTIONAL STRUCTURE? OR 3D STRUCTURE?) AND PHYTASE AND  
(MUTANT? OR MODIFIED OR VARIANT? OR MUTAION?)

=> s (3-dimentional structure? or 3D structure?) and phytase and (mutant? or modified or v  
L3 2 (3-DIMENTIONAL STRUCTURE? OR 3D STRUCTURE?) AND PHYTASE AND  
(MUTANT? OR MODIFIED OR VARIANT? OR MUTATION?)

=> dup rem l3  
PROCESSING COMPLETED FOR L3  
L4 2 DUP REM L3 (0 DUPLICATES REMOVED)

=> d l4 1-2 ibib ab

L4 ANSWER 1 OF 2 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on  
STN  
ACCESSION NUMBER: 2000:577138 SCISEARCH  
THE GENUINE ARTICLE: 337RU  
TITLE: Optimization of the catalytic properties of *Aspergillus*  
*fumigatus* **phytase** based on the three-dimensional  
structure  
AUTHOR: Tomschy A; Tessier M; Wyss M; Brugger R; Broger C;  
Schnoebelen L; VanLoon A P G M; Pasamontes L (Reprint)  
CORPORATE SOURCE: ROCHE VITAMINS INC, BLDG 102, 340 KINGSLAND ST, NUTLEY, NJ  
07110 (Reprint); F HOFFMANN LA ROCHE & CO LTD, CH-4072  
BASEL, SWITZERLAND  
COUNTRY OF AUTHOR: USA; SWITZERLAND  
SOURCE: PROTEIN SCIENCE, (JUL 2000) Vol. 9, No. 7, pp. 1304-1311.  
Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW  
YORK, NY 10011-4211.  
ISSN: 0961-8368.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 16

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Previously, we determined the DNA and amino acid sequences as well as  
biochemical and biophysical properties of a series of fungal phytases. The  
amino acid sequences displayed 49-68% identity between species, and the  
catalytic properties differed widely in terms of specific activity,  
substrate specificity, and pH optima. With the ultimate goal to combine  
the most favorable properties of all phytases in a single protein, we  
attempted, in the present investigation, to increase the specific activity  
of *Aspergillus fumigatus* **phytase**. The crystal structure of  
*Aspergillus niger* NRRL 3135 **phytase** known at 2.5 Angstrom  
resolution served to specify all active site residues. A multiple amino  
acid sequence alignment was then used to identify nonconserved active site  
residues that might correlate with a given favorable property of interest.  
Using this approach, Gln27 of *A. fumigatus* **phytase** (amino acid  
numbering according to *A. niger* **phytase**) was identified as  
likely to be involved in substrate binding and/or release and, possibly,  
to be responsible for the considerably lower specific activity (26.5 vs.  
196 U . [mg protein]<sup>-1</sup>) at pH 5.0) of *A. fumigatus* **phytase** when  
compared to *Aspergillus terreus* **phytase**, which has a Leu at the  
equivalent position. Site-directed mutagenesis of Gln27 of *A. fumigatus*  
**phytase** to Leu in fact increased the specific activity to 92.1 U .  
(mg protein)<sup>-1</sup>, and this and other **mutations** at position 27  
yielded an interesting array of pH activity profiles and substrate  
specificities. Analysis of computer models of enzyme-substrate complexes  
suggested that Gln27 of wild-type *A. fumigatus* **phytase** forms a  
hydrogen bond with the 6-phosphate group of myo-inositol hexakisphosphate,  
which is weakened or lost with the amino acid substitutions tested. If  
this hydrogen bond were indeed responsible for the differences in specific  
activity, this would suggest product release as the rate-limiting step of  
the *A. fumigatus* wild-type **phytase** reaction.

L4 ANSWER 2 OF 2 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN  
ACCESSION NUMBER: 1999-01149 BIOTECHDS  
TITLE: New **modified** protease with increased activity;  
recombinant enzyme production by vector expression in host  
cell and application in food industry  
AUTHOR: Kostrewa D; Pasamontes L; Tomschy A; van Loon A; Vogel K;

Wyss M  
PATENT ASSIGNEE: Roche  
LOCATION: Basle, Switzerland.  
PATENT INFO: AU 9859543 1 Oct 1998  
APPLICATION INFO: AU 1998-59543 25 Mar 1998  
PRIORITY INFO: EP 1997-810175 25 Mar 1997  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 1998-595453 [51]

AB A process for the production of a **modified phytase** with improved activity properties is new and involves: modelling the three-dimensional (3D) **structure** of the **phytase** (preferably from *Aspergillus fumigatus*) being **modified**, and optionally that of another **phytase** (preferably from *Aspergillus terreus* or *Aspergillus niger*) with better activity, on the basis of the **3D-structure** of *Aspergillus niger phytase*; identifying the amino acids in the active site that differ between the two structures; changing at least one of the differing amino acids in the active site in the **phytase** and incorporating the DNA molecule into an expression vector; and using the DNA or the vector to transform a host cell which is then cultured and isolated. Also claimed are: the **phytase** produced; vectors containing the **modified phytase** DNA; host cells containing the DNA or vector; and feed or foods containing the **modified phytase**. Phytases are useful as feed additives for converting **phytase** to myoinositol and inorganic phosphate. The addition of a **modified phytase** to feed reduces the amount of phytate in animal manure. (118pp)

=> d his

(FILE 'HOME' ENTERED AT 14:46:21 ON 02 FEB 2005)

FILE 'MEDLINE, HCAPLUS, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH' ENTERED AT 14:47:26 ON 02 FEB 2005

L1 1 S (3-DIMENTIONAL STRUCTURE? OR 3D STRUCTURE?) AND (PHYTASE AND  
L2 1 S (3-DIMENTIONAL STRUCTURE? OR 3D STRUCTURE?) AND PHYTASE AND  
L3 2 S (3-DIMENTIONAL STRUCTURE? OR 3D STRUCTURE?) AND PHYTASE AND  
L4 2 DUP REM L3 (0 DUPLICATES REMOVED)

=> log y

COST IN U.S. DOLLARS

SINCE FILE

TOTAL

ENTRY

SESSION

FULL ESTIMATED COST

40.29

40.71

STN INTERNATIONAL LOGOFF AT 14:59:41 ON 02 FEB 2005

|   |            |         |
|---|------------|---------|
| => file medline hcaplus embase biosis biotechds scisearch |            |         |
| COST IN U.S. DOLLARS                                      | SINCE FILE | TOTAL   |
|   | ENTRY      | SESSION |
| FULL ESTIMATED COST                                       | 0.21       | 0.21    |

FILE 'MEDLINE' ENTERED AT 15:00:42 ON 02 FEB 2005

FILE 'HCAPLUS' ENTERED AT 15:00:42 ON 02 FEB 2005

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FILE 'EMBASE' ENTERED AT 15:00:42 ON 02 FEB 2005

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FILE 'BIOSIS' ENTERED AT 15:00:42 ON 02 FEB 2005

Copyright (c) 2005 The Thomson Corporation.

FILE 'BIOTECHDS' ENTERED AT 15:00:42 ON 02 FEB 2005

COPYRIGHT (C) 2005 THE THOMSON CORPORATION

FILE 'SCISEARCH' ENTERED AT 15:00:42 ON 02 FEB 2005

Copyright (c) 2005 The Thomson Corporation.

=> s (3D structure or 3 dimensional structure) and Aspergillus phytase?

L1 1 (3D STRUCTURE OR 3 DIMENSIONAL STRUCTURE) AND ASPERGILLUS PHYTASE?

=> s (3D structure or 3 dimensional structure) and phytase?

L2 14 (3D STRUCTURE OR 3 DIMENSIONAL STRUCTURE) AND PHYTASE?

=> dup rem l2

PROCESSING COMPLETED FOR L2

L3 9 DUP REM L2 (5 DUPLICATES REMOVED)

=> d l3 1-9 ibib ab

L3 ANSWER 1 OF 9 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

ACCESSION NUMBER: 2004:869668 SCISEARCH

THE GENUINE ARTICLE: 855JN

TITLE: Crystallographic snapshots of Aspergillus fumigatus **phytase**, revealing its enzymatic dynamics

AUTHOR: Liu O; Huang Q Q; Lei X G; Hao Q (Reprint)

CORPORATE SOURCE: Cornell Univ, Dept Mol Biol & Genet, Ithaca, NY 14853 USA (Reprint); Cornell Univ, MacCHESS, Ithaca, NY 14853 USA; Cornell Univ, Dept Anim Sci, Ithaca, NY 14853 USA

COUNTRY OF AUTHOR: USA

SOURCE: STRUCTURE, (SEP 2004) Vol. 12, No. 9, pp. 1575-1583. Publisher: CELL PRESS, 1100 MASSACHUSETTS AVE, CAMBRIDGE, MA 02138 USA.

ISSN: 0969-2126.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 30

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Understanding of the atomic movements involved in an enzymatic reaction needs structural information on the active and inactive native enzyme molecules and on the enzyme-substrate, enzyme-intermediate, and enzyme-product(s) complexes. By using the X-ray crystallographic method, four crystal structures of Aspergillus fumigatus **phytase** were obtained at resolution higher than 1.7 Angstrom. The pH-dependent catalytic activity of A. fumigatus **phytase** was linked to three water molecules that may prevent the substrate from binding and thus block nucleophilic attack of the catalytic imidazole nitrogen. Comparison of

various structures also identified the water molecule that attacks the phosphamide bond during the hydrolysis process, and established the hydrolysis pathway of the intermediate. Additionally, two reaction product phosphates were observed at the active site, suggesting a possible product release pathway after hydrolysis of the intermediate. These results can help explain the catalytic mechanism throughout the whole acid phosphatase family, as all key residues are conserved.

L3 ANSWER 2 OF 9 MEDLINE on STN DUPLICATE 1  
 ACCESSION NUMBER: 2003305590 MEDLINE  
 DOCUMENT NUMBER: PubMed ID: 12804608  
 TITLE: Fungal phyA gene expressed in potato leaves produces active and stable **phytase**.  
 AUTHOR: Ullah Abul H J; Sethumadhavan Kandan; Mullaney Edward J; Ziegelhoffer Thomas; Austin-Phillips Sandra  
 CORPORATE SOURCE: Southern Regional Research Center, ARS, USDA, 1100 Robert E. Lee Boulevard, New Orleans, LA 70124, USA..  
 aullah@srcc.ars.usda.gov  
 SOURCE: Biochemical and biophysical research communications, (2003 Jun 27) 306 (2) 603-9.  
 Journal code: 0372516. ISSN: 0006-291X.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 200307  
 ENTRY DATE: Entered STN: 20030702  
 Last Updated on STN: 20030726  
 Entered Medline: 20030725

AB Fungal phyA gene from *Aspergillus ficuum* (niger) was cloned and expressed in potato leaves. The recombinant enzyme was stable and catalytically active. The expressed protein in the leaves of the dicotyledonous plant retained most physical and catalytic properties of the benchmark *A. ficuum* **phytase**. The expressed enzyme was, however, 15% less glycosylated than the native **phytase**. The usual bi-hump pH optima profile, which is characteristic of the fungal **phytase**, was altered; however, the pH optimum at 5.0 was unchanged for phytate and at 4.0 for synthetic substrate p-nitrophenyl phosphate. The temperature was, however, unchanged. The expressed **phytase** was found to be as sensitive as the native enzyme to the inhibitory action of pseudo substrate, myo-inositol hexasulfate, while losing about 90% of the activity at 20 microm inhibitor concentration. Similar to the benchmark **phytase**, the expressed **phytase** in leaves was completely inactivated by Arg modifier phenylglyoxal at 60 nM. In addition, the expressed **phytase** in the leaves was inhibited by antibody raised against a 20-mer internal peptide, which is present on the surface of the molecule as shown by the X-ray deduced 3D structure of fungal **phytase**. Taken together, the biochemical evidences indicate that fungal **phytase** when cloned and expressed in potato leaves produces a stable and active biocatalyst. 'Biofarming,' therefore, is an alternative way to produce functional hydrolytic enzymes as exemplified by the expression of *A. ficuum* (niger) phyA gene in potato leaf.

L3 ANSWER 3 OF 9 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.  
 on STN  
 ACCESSION NUMBER: 2003080758 EMBASE  
 TITLE: Beta-propellers: Associated functions and their role in human diseases.  
 AUTHOR: Pons T.; Gomez R.; China G.; Valencia A.  
 CORPORATE SOURCE: T. Pons, Ctr. Ingenieria Genet. Biotecnologia, P.O. Box 6162, Havana 10600, Cuba. tirso.pons@cigb.edu.cu  
 SOURCE: Current Medicinal Chemistry, (2003) 10/6 (505-524).  
 Refs: 239  
 ISSN: 0929-8673 CODEN: CMCHE7

COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; General Review  
FILE SEGMENT: 005 General Pathology and Pathological Anatomy  
029 Clinical Biochemistry  
030 Pharmacology  
037 Drug Literature Index

LANGUAGE: English  
SUMMARY LANGUAGE: English

AB The .beta.-propeller fold appears as a very fascinating architecture based on four-stranded antiparallel and twisted .beta.-sheets, radially arranged around a central tunnel. Similar to the .alpha./beta.-barrel (TIM-barrel) fold, the .beta.-propeller has a wide range of different functions, and is gaining substantial attention. Some proteins containing .beta.-propeller domains have been implicated in the pathogenesis of a variety of diseases such as cancer, Alzheimer, Huntington, arthritis, familial hypercholesterolemia, retinitis pigmentosa, osteogenesis, hypertension, and microbial and viral infections. This article reviews some aspects of 3D structure, amino acids sequence regularities, and biological functions of the proteins containing .beta.-propeller domains. Major emphasis has been laid on .beta.-propellers whose functions are associated to human diseases. Recent research efforts reported in the fields of protein engineering, drug design, and protein structure-function relationship studies, concerning the .beta.-propeller architecture, have also been discussed.

L3 ANSWER 4 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2001:816739 HCAPLUS  
DOCUMENT NUMBER: 135:356734  
TITLE: Protein engineering for reduced allergenicity  
INVENTOR(S): Roggen, Erwin Ludo; Ernst, Steffen; Svendsen, Allan; Friis, Esben Peter; Von Der Osten, Claus  
PATENT ASSIGNEE(S): Novozymes A/S, Den.  
SOURCE: PCT Int. Appl., 513 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 3  
PATENT INFORMATION:

| PATENT NO.             | KIND   | DATE     | APPLICATION NO. | DATE       |
|------------------------|--|----------|-----------------|------------|
| WO 2001083559          | A2   | 20011108 | WO 2001-DK293   | 20010430   |
| WO 2001083559          | A3   | 20020620 |                 |            |
| W:                     | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM |          |                 |            |
| RW:                    | GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG   |          |                 |            |
| CA 2406621             | AA   | 20011108 | CA 2001-2406621 | 20010430   |
| EP 1280817             | A2   | 20030205 | EP 2001-927643  | 20010430   |
| R:                     | AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR   |          |                 |            |
| PRIORITY APPLN. INFO.: |  |          | DK 2000-707     | A 20000428 |
|                        |  |          | US 2000-203345P | P 20000510 |
|                        |  |          | DK 2001-327     | A 20010228 |
|                        |  |          | US 2001-277817P | P 20010321 |
|                        |  |          | WO 2001-DK293   | W 20010430 |

AB The authors disclose a method of selecting a protein variant having modified immunogenicity as compared to the parent protein. The method comprises (1) obtaining antibody-binding peptide sequences; (2) using the sequences to localize epitopes on the 3-dimensional



structure of parent protein; (3) defining an epitope area including amino acids situated within 5.ANG.; (4) changing one or more of the epitope amino acids by genetic engineering of mutations in the DNA sequence encoding the parent protein, and (5) expressing the engineered protein variant and evaluating the immunogenicity. In one example, linear and discontinuous epitopes of savinase were mapped, mutants engineered, and their immunogenicity examd. In a second example, epitopes of environmental allergens (e.g., dust mite, pollen, venom) and com. enzymes (e.g., subtilisin, carezyme, laccase, amylase) were mapped.

L3 ANSWER 5 OF 9 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2001-06291 BIOTECHDS

TITLE: Thermostable **phytase** enzyme having a 2.1 Angstroms crystal structure having a propeller shape, useful in feed production for degrading phytate regardless of the position of its phosphate groups;

for use in protein engineering and phytate degradation

AUTHOR: Oh T K; Oh B C; Ha N C; Choi Y W; Lee D K; Oh B H

PATENT ASSIGNEE: Korea-Res.Inst.Biosci.Biotechnol.; Daesung-Microbiol.Lab.

LOCATION: Daejon, Korea; Kyungki-do, Korea.

PATENT INFO: WO 2001004275 18 Jan 2001

APPLICATION INFO: WO 2000-KR2 4 Jan 2000

PRIORITY INFO: KR 1999-27826 9 Jul 1999

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2001-138336 [14]

AB A thermostable **phytase** derived from *Bacillus amyloliquefaciens* DS-11 with a 2.1 A crystal structure of a propeller shape comprising 6 eternal blades each consisting of 4 or 5 anti-parallel beta-strands, is claimed. The calcium binding sites consist of 3 high-affinity calcium binding sites and 3 low-affinity calcium binding sites located inside the 6 blades. The calcium ion binding motifs of the enzyme are useful in synthesizing highly thermostable proteins. Elucidation of active sites of the enzyme from a 3D **structure** can help design new enzymes having those sites with the aid of recent advanced technology of protein engineering. The **phytase** is useful in feed production for degrading phytate regardless of the position of its phosphate groups. The **phytase** can degrade phytate regardless of the position of phosphate groups in a phytate during feed production and the enzyme activity can be maintained at a high temp. The enzyme has 6 blades in the form of a propeller with the fourth strand connected to the fifth blade. (17pp)

L3 ANSWER 6 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 2000:314711 HCAPLUS

DOCUMENT NUMBER: 132:333397

TITLE: Engineering of enzymes for reduced allergenicity

INVENTOR(S): Roggen, Erwin Ludo; Olsen, Arne Agerlin; Ernst, Steffen

PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.

SOURCE: PCT Int. Appl., 91 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO.    | KIND  | DATE     | APPLICATION NO. | DATE     |
|---------------|---|----------|-----------------|----------|
| -----         | ----  | -----    | -----           | -----    |
| WO 2000026230 | A1  | 20000511 | WO 1999-DK541   | 19991012 |
| W:            | AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, |          |                 |          |

BY, KG, KZ, MD, RU, TJ, TM  
 RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE,  
 DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF,  
 CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

|  |    |          |                 |          |
|--|----|----------|-----------------|----------|
| CA 2348938   | AA | 20000511 | CA 1999-2348938 | 19991012 |
| AU 9960787   | A1 | 20000522 | AU 1999-60787   | 19991012 |
| AU 776534  | B2 | 20040916 |                 |          |
| EP 1124843   | A1 | 20010822 | EP 1999-947260  | 19991012 |
| EP 1124843   | B1 | 20040407 |                 |          |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,<br>IE, SI, LT, LV, FI, RO |    |          |                 |          |
| JP 2003500003  | T2 | 20030107 | JP 2000-579618  | 19991012 |
| EP 1277762   | A2 | 20030122 | EP 2002-20745   | 19991012 |
| EP 1277762   | A3 | 20030409 |                 |          |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,<br>IE, FI, CY             |    |          |                 |          |
| NZ 511291  | A  | 20030829 | NZ 1999-511291  | 19991012 |
| AT 263778  | E  | 20040415 | AT 1999-947260  | 19991012 |
| US 6686164   | B1 | 20040203 | US 1999-417608  | 19991013 |
| US 2004175757  | A1 | 20040909 | US 2003-730454  | 20031208 |

PRIORITY APPLN. INFO.:

|  |                 |    |          |
|--|-----------------|----|----------|
|  | DK 1998-1402    | A  | 19981030 |
|  | DK 1998-1645    | A  | 19981125 |
|  | DK 1999-1417    | A  | 19991004 |
|  | US 1998-107165P | P  | 19981105 |
|  | US 1998-111386P | P  | 19981208 |
|  | US 1999-157429P | P  | 19991004 |
|  | EP 1999-947260  | A3 | 19991012 |
|  | WO 1999-DK541   | W  | 19991012 |
|  | US 1999-417608  | A1 | 19991013 |

AB The authors disclose methodol. for selecting a protein variant having reduced immunogenicity as compared to the parent protein. The methodol. comprises screening a random peptide display library with antibodies raised against the protein of interest, sequencing the peptides, or the DNA sequence encoding the peptides, and identifying epitope patterns by sequence alignment. The epitope patterns are then located on the **3-dimensional structure** of the parent protein and are used to define an area including amino acids situated within 5 .ANG. from the linear epitope amino acids. By genetic engineering mutations of the DNA sequence encoding the parent protein without impairing functionality of the protein are constructed and evaluated for reduced immunogenicity. In one example, the authors generated the enzyme Savinase with an R170F mutation. In competitive IgE ELISA, this variant was less effective in competing for anti-Savinase antibodies. In addn., the Savinase variant exhibited an 80% redn. in the prodn. of specific IgE in mice.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 7 OF 9 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation. on STN

ACCESSION NUMBER: 2000:577138 SCISEARCH  
 THE GENUINE ARTICLE: 337RU  
 TITLE: Optimization of the catalytic properties of *Aspergillus fumigatus* **phytase** based on the three-dimensional structure  
 AUTHOR: Tomschy A; Tessier M; Wyss M; Brugger R; Broger C; Schnoebelen L; VanLoon A P G M; Pasamontes L (Reprint)  
 CORPORATE SOURCE: ROCHE VITAMINS INC, BLDG 102, 340 KINGSLAND ST, NUTLEY, NJ 07110 (Reprint); F HOFFMANN LA ROCHE & CO LTD, CH-4072 BASEL, SWITZERLAND  
 COUNTRY OF AUTHOR: USA; SWITZERLAND  
 SOURCE: PROTEIN SCIENCE, (JUL 2000) Vol. 9, No. 7, pp. 1304-1311. Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW YORK, NY 10011-4211. ISSN: 0961-8368.

DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 16

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Previously, we determined the DNA and amino acid sequences as well as biochemical and biophysical properties of a series of fungal **phytases**. The amino acid sequences displayed 49-68% identity between species, and the catalytic properties differed widely in terms of specific activity, substrate specificity, and pH optima. With the ultimate goal to combine the most favorable properties of all **phytases** in a single protein, we attempted, in the present investigation, to increase the specific activity of *Aspergillus fumigatus* **phytase**. The crystal structure of *Aspergillus niger* NRRL 3135 **phytase** known at 2.5 Angstrom resolution served to specify all active site residues. A multiple amino acid sequence alignment was then used to identify nonconserved active site residues that might correlate with a given favorable property of interest. Using this approach, Gln27 of *A. fumigatus* **phytase** (amino acid numbering according to *A. niger* **phytase**) was identified as likely to be involved in substrate binding and/or release and, possibly, to be responsible for the considerably lower specific activity (26.5 vs. 196 U . [mg protein]<sup>-1</sup>) at pH 5.0) of *A. fumigatus* **phytase** when compared to *Aspergillus terreus* **phytase**, which has a Leu at the equivalent position. Site-directed mutagenesis of Gln27 of *A. fumigatus* **phytase** to Leu in fact increased the specific activity to 92.1 U . (mg protein)<sup>-1</sup>, and this and other mutations at position 27 yielded an interesting array of pH activity profiles and substrate specificities. Analysis of computer models of enzyme-substrate complexes suggested that Gln27 of wild-type *A. fumigatus* **phytase** forms a hydrogen bond with the 6-phosphate group of myo-inositol hexakisphosphate, which is weakened or lost with the amino acid substitutions tested. If this hydrogen bond were indeed responsible for the differences in specific activity, this would suggest product release as the rate-limiting step of the *A. fumigatus* wild-type **phytase** reaction.

L3 ANSWER 8 OF 9 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

ACCESSION NUMBER: 1999-01149 BIOTECHDS

TITLE: New modified protease with increased activity;  
recombinant enzyme production by vector expression in host  
cell and application in food industry

AUTHOR: Kostrewa D; Pasamontes L; Tomschy A; van Loon A; Vogel K;  
Wyss M

PATENT ASSIGNEE: Roche

LOCATION: Basle, Switzerland.

PATENT INFO: AU 9859543 1 Oct 1998

APPLICATION INFO: AU 1998-59543 25 Mar 1998

PRIORITY INFO: EP 1997-810175 25 Mar 1997

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 1998-595453 [51]

AB A process for the production of a modified **phytase** with improved activity properties is new and involves: modelling the three-dimensional (3D) **structure** of the **phytase** (preferably from *Aspergillus fumigatus*) being modified, and optionally that of another **phytase** (preferably from *Aspergillus terreus* or *Aspergillus niger*) with better activity, on the basis of the 3D-**structure** of *Aspergillus niger* **phytase**; identifying the amino acids in the active site that differ between the two structures; changing at least one of the differing amino acids in the active site in the **phytase** and incorporating the DNA molecule into an expression vector; and using the DNA or the vector to transform a host cell which is then cultured and isolated. Also claimed are: the **phytase** produced; vectors containing the modified **phytase** DNA; host cells containing the DNA or vector;

and feed or foods containing the modified **phytase**.  
**Phytases** are useful as feed additives for converting  
**phytase** to myoinositol and inorganic phosphate. The addition of  
a modified **phytase** to feed reduces the amount of phytate in  
animal manure. (118pp)

L3 ANSWER 9 OF 9 HCAPLUS COPYRIGHT 2005 ACS on STN  
ACCESSION NUMBER: 1996:626316 HCAPLUS  
DOCUMENT NUMBER: 125:269217  
TITLE: Disulfide bonds are necessary for structure and  
activity in *Aspergillus ficuum* **phytase**  
AUTHOR(S): Ullah, Abul H. J.; Mullaney, Edward J.; Mullaney,  
Edward J.  
CORPORATE SOURCE: Southern Regional Research Center, ARS, USDA, New  
Orleans, LA, 70124, USA  
SOURCE: Biochemical and Biophysical Research Communications  
(1996), 227(2), 311-317  
CODEN: BBRC9; ISSN: 0006-291X  
PUBLISHER: Academic  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The function of disulfide bonds in *A. ficuum* **phytase** (I) was  
elucidated by unfolding studies, using guanidine-HCl (GuHCl) as  
denaturant. Although I was totally inactivated by 0.8M GuHCl, at pH 5.0,  
the active conformation was instantaneously restored by 0.6M GuHCl at pH  
5.0. Conditions which would permit refolding of I were completely negated  
by 10 mM .beta.-mercaptoethanol and caused its catalytic demise at pH 7.5.  
Assay of free SH groups using Ellman's reagent indicated that none of the  
SH groups in the 10 Cys residues in I were free; 5 disulfide bonds were  
predicted for the enzyme. Sequence comparison of fungal I and yeast acid  
phosphatases indicated 4 conserved Cys residues. Thus, disulfide bonds  
play an important role in the folding of fungal I; any perturbation of the  
process of its formation causes an altered 3-dimensional  
**structure** that is inconsistent with catalytic activity.

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FILE 'MEDLINE, HCAPLUS, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH' ENTERED AT  
15:00:42 ON 02 FEB 2005

L1 1 S (3D STRUCTURE OR 3 DIMENSIONAL STRUCTURE) AND ASPERGILLUS PHY  
L2 14 S (3D STRUCTURE OR 3 DIMENSIONAL STRUCTURE) AND PHYTASE?  
L3 9 DUP REM L2 (5 DUPLICATES REMOVED)

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